

CLAIMS

What is claimed is:

1. A method for comparing gene expression level, characterized in that the method includes:
 - (a) labeling mRNA from different sources with a suitable method, and mixing the labeled mRNA fragments equally to obtain a template for polymerase chain reaction (PCR);
 - (b) performing a polymerase chain reaction using source-specific primers and a gene-specific primer; and
 - (c) detecting a sequence of amplified DNA fragments with bioluminescence analysis, a base type and a signal intensity in a sequencing profile representing a gene source and a relative expression level, respectively.
2. The method for comparing gene expression level according to claim 1, wherein the mRNA from different sources is an expressed mRNA of a given gene from different individuals of a species, or is an expressed mRNA of a given gene from different organs of an individual, or is an expressed mRNA of a given gene of a same species at different states of chemical stimulation or physical stimulation.
3. The method for comparing gene expression level according to claim 1, wherein the source-specific primers include an identical base species and base number but a different base order, each primer representing a gene source.
4. The method for comparing gene expression level according to claim 1, wherein the suitable method is a method to distinguish a gene source by a DNA fragment with a suitable length,

a first of the method including:

performing a reverse transcription-polymerase chain reaction (RT-PCR) to obtain complementary DNA (cDNA) fragments of a given gene in each source;

digesting cDNA into fragments with a suitable length using a restriction endonuclease; and

ligating each of the digested cDNA fragments with a selective adapter, a different adapter corresponding to mRNA from a different source;

a second of the method including:

synthesizing a first strand of complementary DNA (cDNA) fragments of mRNA samples from each source using polythymine primers fixed on microsphere's surface; and

synthesizing a complementary second strand of cDNA using anchored primers containing sequences corresponding to gene sources in a 5'-terminal region, a 5'-terminal region being used for identifying different sources of a given gene;

and a third of the method including:

preparing a first strand of the complementary DNA (cDNA) fragments of mRNA samples from each source by directly hybridizing anchored primers containing sequences corresponding to gene sources in a 5'-terminal region with mRNA; and

constructing of a 5'-terminal region of the anchored primers the same as that in the second of the method.

5. The method for comparing gene expression level according to claim 4, wherein the selective adapter is a cuneal dsDNA (double strand DNA) containing a part of sequences complementary to recognition sequences of the restriction

endonuclease and can be fully ligated with restriction enzyme cutting ends in DNA fragment by a DNA ligase, a 5' terminal region of one of the strands in the adapter containing a sequence specific to gene sources, a 3' terminal region of the other strand in the adapter containing bases non-complementary to a opposite strand, or a 3' end of the other strand in the adapter being modified to block ability of extension reaction by DNA polymerase, and the adapter having a structure of a "Y" shape consisting of two strands, one end of the adapter being divided into two branches due to no complementary bases, and the other end being formed of a shape of restriction enzyme cutting site.

6. The method for comparing gene expression level according to claim 4, wherein the part used for identifying gene sources in selective adapters and anchored primers includes identical base species and base number but different base order, each of the selective adapters having a same melting temperature, and each of the anchored primers including a same melting temperature.
7. The method for comparing gene expression level according to claim 1, wherein the bioluminometric assay is based on a quantitative determination of pyrophosphate released from an extension reaction.
8. The method for comparing gene expression level according to claim 7, wherein the extension reaction is polymerization of single-stranded PCR products annealed with a given primer or primer mixtures by DNA polymerase when a deoxynucleotide (dNTP) added in a given order, or a dideoxynucleotide (ddNTP) added in a given order, or an analog of dNTP or ddNTP added in a given order is complementary to the template.
9. The method for comparing gene expression level according to claim 8, wherein the single-stranded PCR products are obtained by treating the PCR products of

claim 1 with a physical method or a chemical method, the physical method being to use a biotinylated primer for PCR amplification and then to prepare single-stranded DNAs by a solid phase method, and the chemical method being to use an enzyme for the digestion to prepare single-stranded DNAs.

10. The method for comparing gene expression level according to claim 7, wherein the extension reaction is polymerization of the PCR products of claim 1 treated by enzymes to degrade PPi produced during PCR reaction, excess dNTPs and excess primers, a single-strand binding protein (e.g. SSB) being added into the treated PCR products, the rest being performed in accordance with claim 8.